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Research Article

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Biotechnological Potential of the Vetom Series Preparations for the Production of Functional Food Products

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Abstract

The purpose of the study is to determine the biotechnological potential of microorganism strains included in the composition of nutritional supplements Vetom 1.1, Vetom 2, Vetom 3.22 for the production of functional food products based on the in vitro study of their resistance to adverse factors of the human gastrointestinal tract. The results of studies that reproduce individual conditions of the gastrointestinal tract and food products, such as high acidity, concentrations of phenol challenging for bacteria, and the presence of bile, made it possible to establish a high survival rate of microorganism strains, the dynamics of their growth, and the number of viable cells when exposed to a high concentration of sucrose and in various temperature ranges, which suggests that they are promising, especially the *B. subtilis* strain VKPM B 10641 which has the highest adaptive potential.

Keywords

Microorganism strain, Biotechnological potential, Resistance, Viability, Phenol, Bile, Concentration, Probiotics, Functional food

Introduction

The most promising functional food products are products with symbiotic properties containing a complex of pro- and prebiotics. This complex provides a positive effect on the microbiome of the human body by suppressing pathogenic microflora and accelerating the production of substances useful for the body, such as enzymes, vitamins, and amino acids [1-3].

For probiotics, such indicators as the ability to actively compete with pathogenic microflora, the viability in the human body and food systems, high growth and reproduction rates, as well as the absence of signs of pathogenicity and toxicity are valuable from the point of view of their biotechnological potential [4, 5].

We were interested in studying the possibility of using strains of microorganisms used in the production of previously registered and shown to be effective preparations with probiotic properties of the Vetom series as a prebiotic component for the production of enriched functional confectionery.

The study aimed to determine the biotechnological potential of strains of microorganisms included in the composition of nutritional supplements Vetom 1.1, Vetom 2, Vetom 3.22 for the production of functional food products. Our research was aimed at studying the resistance of strains of microorganisms included in the preparations of the Vetom series to unfavorable factors of the human gastrointestinal tract *in vitro*.

Materials and Methods

The objects of the study were preparations of the Vetom series containing strains of microorganisms *Bacillus subtilis* VKPM B 10641, *Bacillus amyloliquefaciens* strain VKPM B-10642 and *Bacillus amyloliquefaciens* strain VKPM B-10643.

Microbiological studies were carried out following the State Standards (GOST) [6-11] and according to modified and special research methods developed by us.

Determination of resistance to phenol

The resistance of the studied bacterial strains to phenol was determined by the change in optical density and the number of CFU/g after 24 hours of culturing the samples at the optimum temperature in a liquid nutrient medium (GRM broth) with a phenol concentration of 0.2%, 0.4%, 0.6%.

Determination of resistance to bile

The cultivation was carried out in a liquid nutrient medium (GRM broth) containing 0.5, 20, and 40% bile for 24 h. In the experiments, we used a preparation of medical bile (Cholemedicata) containing natural bile of cattle. The percentage of survival was assessed by the number of viable bacterial cells in 1 cm^3 of suspension (number of CFU).

Determination of resistance to various concentrations of pH

The cultivation was carried out in a liquid nutrient medium (GRM broth) with a pH of 2, 4, 7, and 9 for 24 h. The percentage of survival was assessed by the number of viable bacterial cells in 1 cm^3 of suspension (number of CFU).

Determination of the growth rate and the number of viable cells was carried out using a culture of the preparation containing 10^2 cells. In test tubes containing 10 ml of the culture medium (GRM broth), 1 ml of the preparation culture was added and incubated at a temperature of 37 °C for 6 hours. Counting was performed after 1, 2, 4, and 6 hours from the start of incubation.

Determination of resistance to high sucrose content

The cultivation was carried out on a liquid nutrient medium (GRM broth) containing 10, 20, 30, 40, 50% sucrose for 24 h at t = (37 ± 1) °C. The percentage of survival was assessed by the number of viable bacterial cells in 1 cm^3 of suspension (number of CFU).

Determination of the survival rate of the studied strains depending on the temperature

The cultivation was carried out on a liquid nutrient medium (GRM broth) for 24 h at a temperature from 20 °C to 90 °C with a step of 10 °C. The percentage of survival was assessed by the number of viable bacterial cells in 1 cm^3 of suspension (number of CFU).

Determination of the effect of the dosage of the grape pomace powder on the growth dynamics of the strain

Determination of the growth rate and the number of viable cells was carried out using a culture of the preparation containing 102 cells. The test preparation of the Vetom series was added to the test tubes containing 10 ml of the culture medium (GRM broth) with the addition of 5% and 10% grape pomace powder and incubated at 37 °C for 48 hours. Counting was performed after 6, 24, 30, and 48 hours from the start of incubation.

The number of viable microorganisms was determined by inoculation and subsequent incubation of tenfold dilutions of the culture liquid in solid media (meat peptone agar, Sabouraud agar).

The experiments were carried out in five-fold repetition, providing reliable results, and the mathematical processing of the experimental results was carried out under the recommendations [12-15].

Results and Discussion

The minimum viable cell count of a probiotic should be at least 10^6 CFU per gram and at least 5×10^7 CFU in a daily serving of such products. This amount should be maintained during the entire shelf life of the product.

Getting into the gastrointestinal tract, most of the preparation cells lose their activity. The human gastrointestinal tract has a rather aggressive environment with high acidity, concentrations of phenol, bile, and enzymes that are challenging for bacteria. The preparation, considered as a potential probiotic component of a functional food product, must show resistance to factors that are characteristic of the human gastrointestinal tract. In this regard, to assess the adaptive potential, we evaluated the resistance of the studied strains to challenging concentrations of phenol and bile and high acidity.

The resistance of the strains of the studied bacteria to phenol is shown in figure 1.

The data shown in the figure indicate that the preparation Vetom 1.1 containing the *Bacillus subtilis* strain VKPM B 10641 is most resistant to the effects of phenol. At all studied

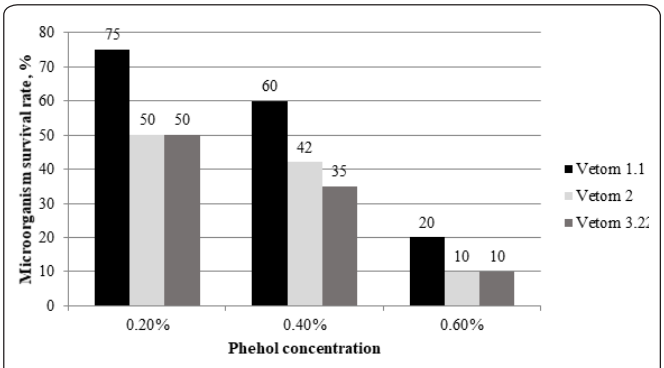

Figure 1: The survival rate of the studied strains in the preparations of the Vetom series in the presence of phenol.

phenol concentrations, this strain showed a fairly high percentage of survival.

The cells of microorganisms are highly sensitive to the bile salts contained in the bile entering the small intestine. In this regard, the resistance of potential probiotics to bile concentration is an important factor that must be evaluated when choosing an effective probiotic. The resistance of microorganisms in preparations of the Vetom series in the presence of bile is shown in figure 2.

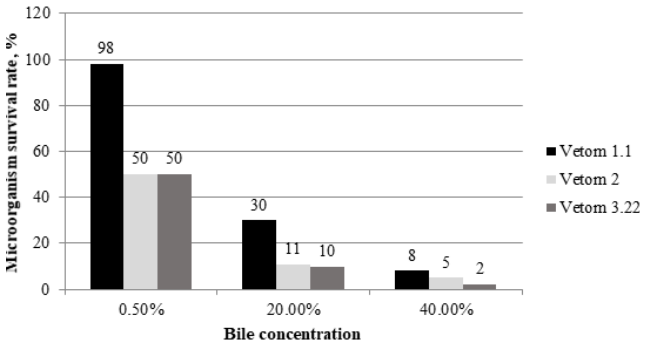

Figure 2: The survival rate of microorganisms in the preparations of the Vetom series in the presence of bile.

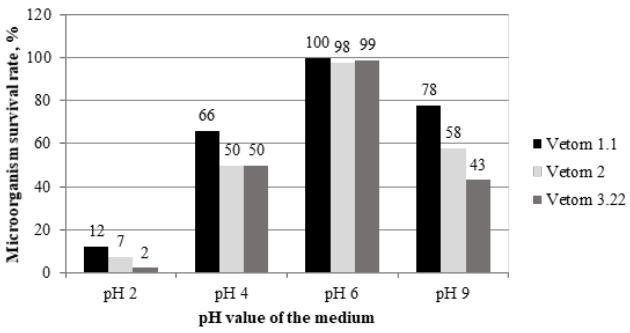

Figure 3: The survival rate of microorganisms in the preparations of the Vetom series at different pH values.

It was found that at a bile concentration of 0.5%, growth stimulation was observed in the *Bacillus subtilis* strain VKPM B 10641 (Vetom 1.1), which is probably due to the activation of the systems responsible for the metabolic processes of bacteria. In the presence of 20 and 40% of bile, a significant decrease in the survival rate of the studied strains was observed. The survival rate at a 20% bile concentration is from 30 to 10%, and at a 40% concentration from 8 to 2%.

The main indicator of the normal functioning of the stomach is its acidity, i. e. the concentration of acid in the gastric juice, measured in pH, the conditional units of the activity of hydrogen ions. Thus, 1 pH is an indicator of maximum acidity, 7 pH is a mark of neutrality, the balance of acid and alkali, and 14 pH is the maximally alkaline medium. It is known that most dry bacteria, getting into the gastrointestinal tract, do not survive. Up to 90% die under the influence of the acidic environment of the stomach and aggressive digestive enzymes. A long time (from 5 to 10 hours) elapses from the moment bacteria of an ordinary dry probiotic enter the body until the beginning of their sanitizing effect. Therefore, it was necessary to study the ability of bacteria to come out of anabiosis and pass into an active state with the least loss.

Figure 3 shows the survival rate of microorganisms in preparations of the Vetom series at different pH values.

For the studied strains, the inhibiting pH value is pH 2.0. The survival rate at this value ranges from 2 to 12%. The *Bacillus subtilis* strain VKPM B 10641 (Vetom 1.1) showed the highest acid resistance. The alkaline environment has the following effect on the strains under study: 40% survival at pH 9.0 in *Bacillus subtilis* VKPM B 10641 (Vetom 1.1), 19% survival in the complex of bacteria Bacillus amyloliquefaciens VKPM B-10642 and Bacillus amyloliquefaciens VKPMB-10643 (Vetom 2), and 7% in bacteria Bacillus amyloliquefaciens VKPM B-10642 (Vetom 3.22). The *Bacillus subtilis* strain VKPM B 10641 (Vetom 1.1) showed the greatest resistance to an alkaline medium.

The prospects of the investigated preparations for the production of probiotic products were assessed taking into account the growth rate and the number of viable cells (Table 2).

Based on table 2, the *B. subtilis* strain VKPM B 10641 showed the highest growth rate and the maximal number of viable cells.

To study the prospects for the use of the studied preparations as a probiotic component in the manufacture of confectionery products, it is necessary to determine the degree of their resistance to high concentrations of sucrose. From literary sources, it is known about the relative tolerance of individual probiotics to small concentrations of sucrose.

To assess the concentration of sucrose for the studied microorganisms, we evaluated their survival rate. Figure 4 shows

Table 1: Characteristics of preparations of the Vetom series

Characteristics	Name of the preparation		
	Vetom 1.1	Vetom 2	Vetom 3.22
Composition	Probiotic microorganisms Bacillus subtilis, recombinant strain VKPM B 10641, corn extract, potato starch, sucrose.	Dry culture of probiotic microorganisms Bacillus amyloliquefaciens strain VKPM B-10642 (DSM 24614), Bacillusamyloliquefaciens strain VKPM B-10643 (DSM 24615), 1x108 CFU/g, 500 mg.	Corn extract fermented with Bacillus amyloliquefaciens microorganisms, strain VKPM B-10642 (DSM 24614).
Release form	Finely dispersed white powder, odorless, soluble in water, with the formation of a white precipitate.		Liquid from light yellow to dark brown, with a specific odor.
Scope of application (description)	The healing effect is provided by the properties of bacteria, which, multiplying in the large intestine, secrete biologically active substances that suppress the growth and development of pathogenic and opportunistic microflora.		
Storage conditions	Relative air humidity not higher than 75% and temperature not higher than 30°C. Shelf life: 4 years from the date of manufacture.		At a temperature of 0-10 °C. Shelf life: 2 years. After opening the bottle, store for no more than 1 week at a temperature of 0 to 10°C.

Table 2: Comparative characteristics of the 6-hour growth rate of the studied microorganisms

Type of preparation/strain	Number of viable cells, CFU/g			
	1 hour	2 hours	4 hours	6 hours
1. Vetom 1.1 B. subtilis VKPM B 10641	11×10^2	56×10^3	21×10^4	67×10^5
2. Vetom 2 B. amyloliquefaciens VKPM B-10642, B. amyloliquefaciens VKPM B-10643	10×10^2	15×10^3	91×10^3	66×10^4
3. Vetom 3.22 B. amyloliquefaciens VKPM B-10642	6×10^2	54×10^2	19×10^3	23×10^4

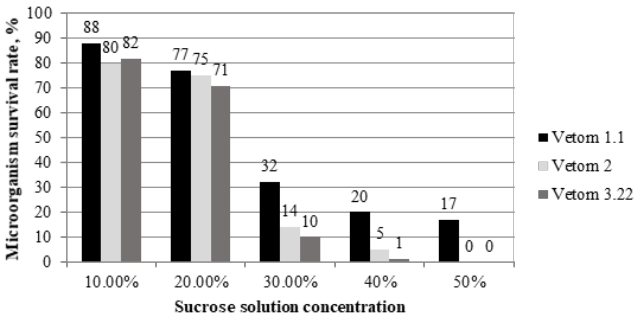

Figure 4: The survival rate of the studied microorganisms in the preparations of the Vetom series in the presence of sucrose.

data on the survival rate of the studied microorganisms in preparations of the Vetom series, depending on the concentration of sucrose.

The data obtained indicate the inhibitory effect of sucrose on the growth rate of the studied strains, while the degree of inhibition of growth increases with an increase in the concentration of sucrose above 20%. The strains *B. amyloliquefaciens* VKPM B-10642 and *B. amyloliquefaciens* VKPM B-10643 (preparations Vetom 2, Vetom 3.22) are more sensitive to an increase in the concentration of sucrose.

The most important indicator characterizing the possibility of using the studied strains as a probiotic component of confectionery products is their thermal stability. In this regard, we studied the survival rate of the strains depending on the temperature. Table 3 shows the survival rate of the studied microorganisms in the preparations of the Vetom series, depending on the temperature.

The data provided in the table allow us to conclude that with an increase in temperature above 50 °C, a decrease in the survival rate of the studied microorganisms is observed. Strain *B. amyloliquefaciens* VKPM — 10642 responds to an increase in temperature to the maximum extent, while strain *B. subtilis* VKPM B 10641 — shows a less expressed reaction.

Considering that this strain is the basis of the Vetom 1.1 preparation, we have chosen this preparation for further research on the development of confectionery products with symbiotic properties.

Table 3: The survival rate of the studied microorganisms of the preparations of the Vetom series, depending on the temperature

Temperature, °C	Preparation/Survival rate, %		
	Vetom 1.1	Vetom 2	Vetom 3.22
20	97	95	96
30	100	100	100
40	100	98	98
50	95	51	47
60	52	27	18
70	40	5	1
80	27	0	0
90	19	0	0

The results of studies that reproduce in vitro certain conditions of the gastrointestinal tract and food products allow us to conclude that the *B. subtilis* strain VKPM B 10641 has a high adaptive potential.

Conclusion

The results of the study indicate a high survival rate of the studied probiotic cultures, the metabolism of which ensures microbiological safety in the production of confectionery products.

Evaluation of the growth rate, the number of viable cells, and resistance to high sucrose concentrations have shown that the studied strains are promising in this regard.

The highest biotechnological potential has been observed in the *B. subtilis* strain VKPM B 10641.

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